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4. TITLE AND SUBTITLE
MEASUREMENT AND REGULATION OF CENTRAL NORADRENERGIC RECEPTORS

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11. SUPPLEMENTARY NOTES

12a DISTRIBUTION/AVAILABILITY STATEMENT
Approved for public release;
distribution unlimited



As proposed in the original application, research this year has concerned studies of the central noradrenergic neuronal system in reactions to stress. We have focused on the role of the noradrenergic system in long-term changes in brain function produced by stress. In previous work we had shown that activation of the noradrenergic system by the sympathomietic drug, yohimbine (YOH), or by stress increases the mRNA levels of the immediate early gene (IEG), c-fos. IEGs serve to regulate the transcription of other genes and may mediate long-term structural and functional changes in the brain during stress. In work done this year, we have shown that lesions of central noradrenergic neurons block the effects of YOH and stress on c-fos. This confirms the importance of norepinephrine (NE) release in the mediation of the central c-fos response. We have also shown that YOH can activate the gene for nerve growth factor (NGF) in the brain. NGF is a neurotrophic agent and may mediate the long-term structural and functional changes produced by noradrenergic activity during stress. With regard to the nature of these long-term effects we have shown that the noradrenergic system has protective actions on neurons in the substantial nigra during the administration of a neurotoxin. Thus protection of neurons from damage may be one of the long-term functions of the noradrenergic system during stress. In addition to these functional studies, we have also made progress on methological problems associated with the measurement of noradrenergic neurotransmission in vivo. We have found that an increase in beta adrenoreceptor activation during stress can be detected from measurement of extra cellular levels of cyclic AMP by micro dialysis. These findings may facilitate future studies of noradrenergic function in vivo during stress.

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# REPORT DOCUMENTATION PAGE



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| | 11 Jan 93 | Annual Tech/1 Dec 91 - 30 Sep 92 |

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**13. ABSTRACT (Maximum 200 words)**

As proposed in the original application, research this year has concerned studies of the central noradrenergic neuronal system in reactions to stress. We have focused on the role of the noradrenergic system in long-term changes in brain function produced by stress. In previous work we had shown that activation of the noradrenergic system by the sympathomietic drug, yohimbine (YOH), or by stress increases the mRNA levels of the immediate early gene (IEG), c-fos. IEGs serve to regulate the transcription of other genes and may mediate long-term structural and functional changes in the brain during stress. In work done this year, we have shown that lesions of central noradrenergic neurons block the effects of YOH and stress on c-fos. This confirms the importance of norepinephrine (NE) release in the mediation of the central c-fos response. We have also shown that YOH can activate the gene for nerve growth factor (NGF) in the brain. NGF is a neurotrophic agent and may mediate the long-term structural and functional changes produced by noradrenergic activity during stress. With regard to the nature of these long-term effects we have shown that the noradrenergic system has protective actions on neurons in the substantial nigra during the administration of a neurotoxin. Thus protection of neurons from damage may be one of the long-term functions of the noradrenergic system during stress. In addition to these functional studies, we have also made progress on methological problems associated with the measurement of noradrenergic neurotransmission in vivo. We have found that an increase in beta adrenoreceptor activation during stress can be detected from measurement of extra cellular levels of cyclic AMP by micro dialysis. These findings may facilitate future studies of noradrenergic function in vivo during stress.

**14. SUBJECT TERMS**

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**Grant Number: AFOSR 89-0208**

**Title: Measurement and regulation of central noradrenergic receptors**

**Professional personnel: Stone, Eric A., Ph.D., Principal investigator**

**Bing, Guoying, M.D., Ph.D., Coinvestigator**

**Zhang, Yi, M.D., Coinvestigator**



**Annual Technical Progress Report**

**Period:** 12/1/91-11/30/92



**Summary:**

As proposed in the original application, research this year has concerned studies of the central noradrenergic neuronal system in reactions to stress. We have focussed on the role of the noradrenergic system in long term changes in brain function produced by stess. In previous work we had shown that activation of the noradrenergic system by the sympathomimetic drug, yohimbine (YOH), or by stress increases the mRNA levels of the immediate early gene (IEG), c-fos. IEGs serve to regulate the transcription of other genes and may mediate long-term structural and functional changes in the brain during stress. In work done this year we have shown that lesions of central noradrenergic neurons block the effects of YOH and stress on c-fos. This confirms the importance of norepinephrine (NE) release in the mediation of the central c-fos response. We have also shown that YOH can activate the gene for nerve growth factor (NGF) in the brain. NGF is a neurotrophic agent and may mediate the long-term structural and functional changes produced by noradrenergic activity during stress. With regard to the nature of these long-term effects we have shown that the noradrenergic system has protective actions on neurons in the substantia nigra during the administration of a neurotoxin. Thus protection of neurons from damage may be one of the long term functions of the noradrenergic system during stress. In addition to these functional studies we have also made progress on methological problems associated with the measurement of noradrenergic neurotransmission in vivo. We have found that an increase in beta adrenoreceptor activation during stress can be detected from measurement of extracellular levels of cyclic AMP by microdialysis. These findings may facilitate future studies of noradrenergic function in vivo during stress.

1) Effect of locus coeruleus lesions on c-fos response to YOH

As noted above, we and other investigators had shown that treatment with YOH, a drug which causes the release of brain NE, produces a marked increase in c-fos mRNA and protein levels in the rat brain (Gubits et al. 1989; Bing et al. 1991a; Bing et al. 1992a). YOH, however, is also known to release dopamine and serotonin as well as NE. To determine to what extent the c-fos response is due to NE release we studied the effect of lesions of the noradrenergic neurons in the locus coeruleus (LC) on the c-fos response to YOH. Rats were unilaterally lesioned in the LC with 6-hydroxydopamine (6-OHDA) and 10 days later given an injection of YOH (5 mg/kg). The animals were perfused 2 hrs after the injection and c-fos protein was assessed immunohistochemically in the frontal cortex as in our previous studies (Bing et al. 1992a). It was found that the lesion totally abolished the c-fos response in the ipsilateral cortex. These results indicate that the noradrenergic system is necessary for the c-fos response to YOH and implicate the release of NE as a primary factor in this process. To determine if these findings also hold for NE release during stress we have conducted preliminary studies on unilaterally lesioned rats subjected to restraint stress. These have yielded similar results. These findings were reported in abstract form (Bing et al. 1991b) and are currently in press (Stone et al. 1992).

2) Effect of YOH on NGF mRNA levels

As noted above, IEGs serve to regulate the transcription of a variety of other genes. One of the genes that has been found to be activated by c-fos is that of NGF (Moccheti et al. 1989). We therefore undertook experiments to determine if brain NE release by YOH increases NGF mRNA in the brain. Rats were injected with YOH or saline and sacrificed at various intervals after (0.5-48 hr). The hippocampus was dissected and processed for RNA as in previous studies. This region of the brain was used because of its high levels of NGF. NGF mRNA was quantified with a ribonuclease protection assay, an extremely sensitive method for mRNA determination. The results are shown in Fig. 1. As can be seen YOH produced a significant increase in hippocampal NGF mRNA levels. The increase peaked at 24-36 hrs post injection at which time there was a 4 fold rise over saline control levels. These results suggest that brain NE release triggers activation of the NGF gene in the CNS. Since NGF has effects on neuronal morphology and viability the present results also suggest that this neurotrophic agent may be one of the factors by which the noradrenergic system produces long-term effects in the CNS. Studies are currently in progress to determine whether NGF mRNA levels are also increased by noradrenergic activation during stress. An abstract of these results has been submitted for presentation (Stone and Bing, In press).

3) Effect of the noradrenergic system on neuronal survival in the substantia nigra

Because of its action on neurotrophic substances, the potentially protective effects of the noradrenergic system has become of interest to us as a possible long-term function during stress. With regard to this function, it was recently found by others that lesions of the LC potentiated the damaging effects of the neurotoxin, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), in the substantia nigra in monkeys (Mavridis et al. 1991). Since this appeared to represent evidence for a trophic effect we chose to replicate the

finding in a more practical animal model involving the mouse. Balb/c mice were given unilateral lesions of the LC with 6-OHDA and 10 days later were administered a subtoxic dose of MPTP (40 mg/kg, i.p. in 4 divided doses over a 8 hr period). After an additional 7 days the animals were perfused for immunohistochemical assay of tyrosine hydroxylase-positive cells in the substantia nigra. It was found that the LC lesion markedly potentiated the effect of the neurotoxin in that significantly fewer intact neurons were found on the lesioned than nonlesioned side (see Figs. 2 and 3). These findings support the notion that the noradrenergic system has trophic actions in the substantia nigra. If this property is confirmed by other techniques it would support the hypothesis that one of the long-term functions of the noradrenergic system during stress is the protection of CNS neurons from damage. These findings were presented in abstract form (Bing et al. 1992b) and are currently being prepared for publication.

4) Methodological advances in the study of noradrenergic neurotransmission

In parallel with the above projects we have also pursued methodological studies aimed at developing new ways to measure noradrenergic neurotransmission in awake behaving rats. One technique that we have been working on is based on the use of microdialysis to detect the cAMP released into the extracellular fluid of the brain in response to beta adrenoceptor stimulation. We have recently found that mild stressors (i.p. saline injection or brief restraint) produce significant increases in cAMP levels in the frontal cortex. Since these stressors are known to release brain NE, these findings suggest that endogenous noradrenergic neurotransmission is being detected. If confirmed by the use of appropriate blocking agents, this will facilitate studies of the behavioral functions of the central noradrenergic system. These findings were presented in abstract form (John and Stone, 1992) and are currently in press (Stone and John, In press).

## Publications and Submissions (12/1/91-pres.)


Stone, E.A., Bing, G., John, S.M., Zhang, Y. & Filer, D. Cellular localization of responses to catecholamines in brain tissue. Prog. Brain Res. 94:303-307, 1992

Stone, E.A., John, S.M., Bing, G. & Zhang, Y. Studies on the cellular localization of biochemical responses to catecholamines in the brain. Brain Res. Bull. 29:285-288, 1992.

Bing, G., Stone, E.A., Zhang, Y. & Filer, D. Immunohistochemical studies of noradrenergic-induced expression of c-fos in the rat CNS. Brain Res., 592:57-62, 1992a.

Bing, G., Chen, S., Zhang, Y., Hillman, D. & Stone, E.A. Noradrenergic-induced expression of c-fos in rat cortex: neuronal localization. Neurosci. Lett. 140:260-264, 1992b.

Stone, E.A. & John, S.M. Stress-induced increase in extracellular levels of cyclic AMP in rat cortex. Brain Res., In press.

Stone, E.A., Zhang, Y., John, S., Filer, D. & Bing, G. Effect of locus

coeruleus lesion on c-fos expression in the cerebral cortex caused by yohimbine injection or stress. Brain Res., In press.

**Interactions** (12/1/91 - pres.)

Hillman, D.E., Chen, S., Aung, T.T., Bing, G. & Stone, E.A. Partial deafferentation of dentate granule cells activates the immediate early gene, c-fos, through alpha-1 and beta adrenergic receptors. Anat. Rec. 232:43A, 1992.

John, S.M. & Stone, E.A. Effect of agents that increase brain norepinephrine (NE) release on brain extracellular cAMP levels. Abstr. Soc. Neurosci. 18:458, 1992

Zhang, Y., Bing, G., Filer, D. & Stone, E.A. Effect of locus coeruleus (LC) lesion on c-fos response to metrazol in rat brain. Abstr. Soc. Neurosci. 18:1375, 1992.

Bing, G., Zhang, Y. & Stone, E.A. Protective action of locus coeruleus noradrenergic system on substantia nigra (SN) of mice treated with MPTP. Abstr. Soc. Neurosci. 18:1375, 1992.

Watanabe, Y., Bing, G., Zhang, Y., McEwen, B.S. & Stone, E.A. Locus coeruleus lesion reduces tyrosine hydroxylase immunoreactivity and mRNA levels in rat substantia nigra. Abstr. Soc. Neurosci. 18:1378, 1992.

Stone, E.A. & Bing, G. Increase in hippocampal NGF mRNA following yohimbine injection. FASEB J., In press.

Stone, E.A. Regulation of noradrenergic neurotransmission. Presented at Louisiana State Univ., Dept. Pharmacology, Jan., 1992.

Stone, E.A. Regulation of noradrenergic neurotransmission. Presented at Mt. Sinai Hospital, Dept. Psychiatry, Dec., 1991.

Stone, E.A. Regulation of noradrenergic neurotransmission. Presented at NINDS, Oct., 1992.

Stone, E.A. Glial cells as targets of the noradrenergic system: implications for trophic actions. Presented in Castres, France, Dec., 1992.

**Literature cited in report**

Bing, G., D. Filer, J. C. Miller, and E. A. Stone. 1991a. Noradrenergic activation of immediate early genes in rat cerebral cortex. *Mol. Brain Res.* 11:43-46.

Gubits, R. M., T. M. Smith, J. L. Fairhurst, and H. Yu. 1989. Adrenergic receptors mediate changes in c-fos mRNA levels in brain. *Mol. Brain Res.* 6:39-45.

Mavridis, M., A-D. Degryse, A. J. Lategan, M. R. Marien, and F. C. Colpaert. 1991. Effects of locus coeruleus lesions on parkinsonian signs, striatal

dopamine and substantia nigra cell loss after 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine in monkeys: a possible role for the locus coeruleus in the progression of parkinson's disease. *Neurosci.* 41:507-523.

Mocchetti, I., M. A. De Bernardi, A. M. Szekely, H. Alho, G. Brooker, and E. Costa. 1989. Regulation of nerve growth factor biosynthesis by beta-adrenergic receptor activation in astrocytoma cells: a potential role of c-fos protein. *Proc. Natl. Acad. Sci.* 86:3891-3895.

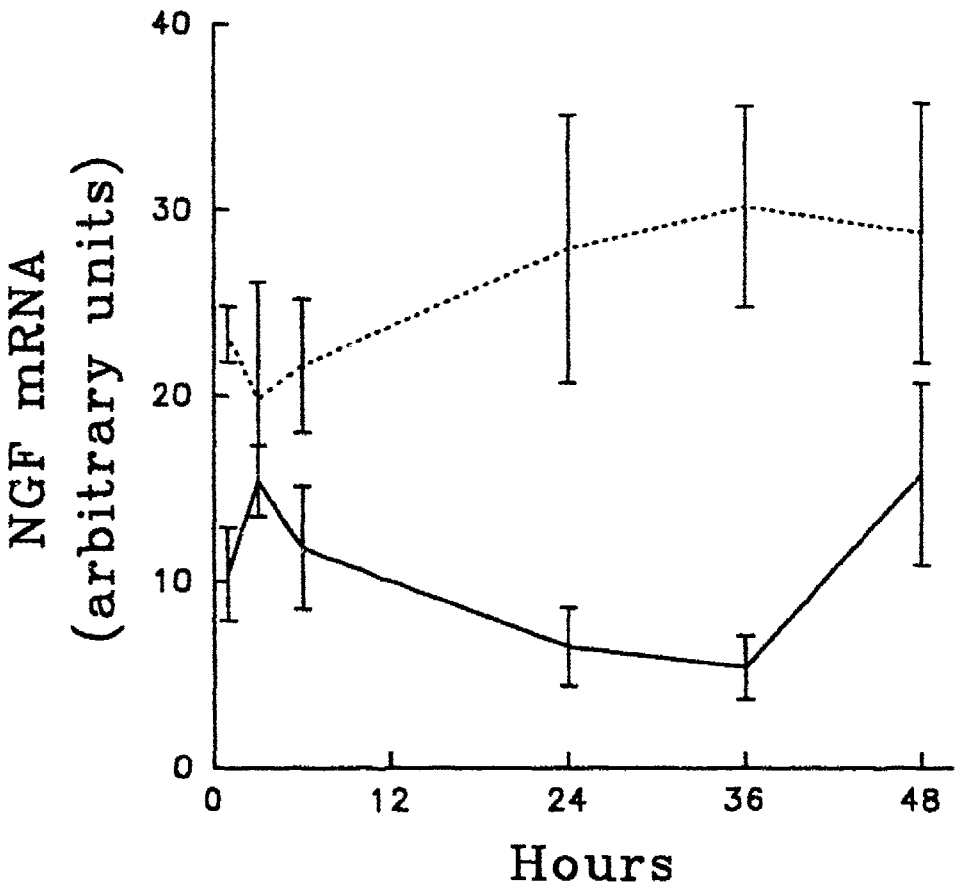


Fig. 1. Effect of injection of yohimbine (5.0 mg/kg, i.p.) (dashed line) or saline (solid line) on levels of nerve growth factor mRNA in rat hippocampus. Each value represents the mean and SEM of 4–8 rats. There was a significant difference between the yohimbine and saline groups at 24 and 36 hrs ($p$'s $< 0.05$).

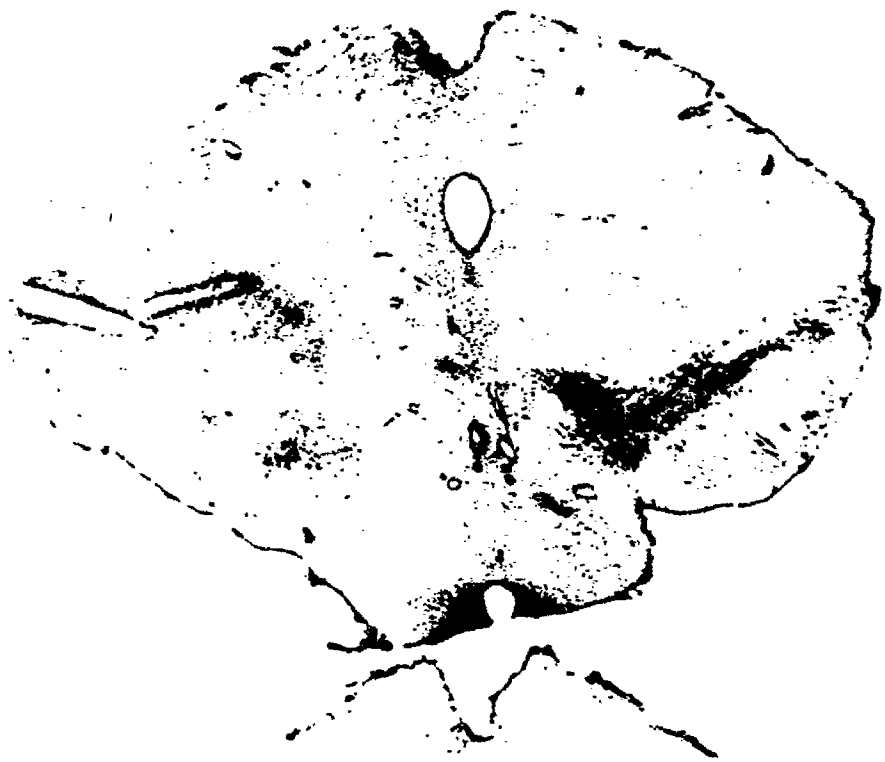

Fig. 2. Tyrosine hydroxylase immunoreactivity (TH-IR) in the locus coeruleus (LC)-lesioned and MPTP-treated mouse substantia nigra. Note marked decrease in TH-IR on side with LC lesion. (left).

Stone, Eric A., PI

BUDGET 12/1/92 - 11/30/93

| Personnel | Role | Time | Percent | Salary | Fringe | Total |
|---|---|---|---|---|---|---|
| Eric A. Stone | PI | 1.0 | 30% | 20,821 | 5747 | 26568 |
| John Manavalan | CoInv | 1.0 | 100% | 26,608 | 7343 | 33951 |

| | | | |
|---|---|---|---|
| Supplies | | | |
| Animals | | 3000 | |
| Isotopes | | 3500 | |
| Glassware | | 3500 | |
| | | | 10000 |
| Other | | | 2000 |
| TOTAL DIRECT COSTS | | | 72,519 |
| INDIRECT COSTS | | | 44,481 |
| total costs | | | 114,000 |

1/7/93

THOMAS A. FITZGERALD,
SENIOR DIRECTOR
GRANTS ADMINISTRATION

DATE